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Free Radical Production and Characterization of Heat-Not-Burn Cigarettes in Comparison to Conventional and Electronic Cigarettes

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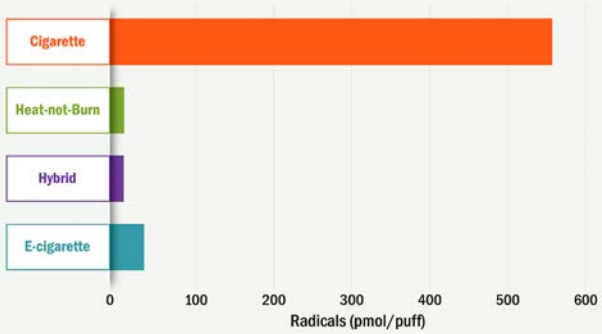
ABSTRACT: With conventional cigarettes, the burning cone reaches temperatures of >900 °C, resulting in the production of numerous toxicants and significant levels of highly reactive free radicals. In attempts to eliminate combustion while still delivering nicotine and flavorings, a newer alternative tobacco product has emerged known as "heat-not-burn" (HnB). These products heat tobacco to temperatures of 250−350 °C depending on the device allowing for the volatilization of nicotine and flavorants while potentially limiting the production of combustion-related toxicants. To better understand how the designs of these new products compare to conventional cigarettes and different styles of electronic cigarettes (e-cigs), we measured and partially characterized their production of free radicals. Smoke or aerosols were trapped by a spin trap phenyl-*N*-*tert*-butylnitrone (PBN) and analyzed for free radicals using electron paramagnetic resonance (EPR). Free radical polarity was assessed by passing the aerosol or smoke through either a polar or nonpolar trap prior to being spin trapped with PBN. Particulate-phase radicals were detected only for conventional cigarettes. Gas-phase free radicals were detected in smoke/aerosol from all products with levels for HnB (IQOS, Glo) (12 pmol/puff) being similar to e-cigs (Juul, SREC, box mod e-cig) and hybrid devices (Ploom) (5−40 pmol/puff) but 50-fold lower than conventional cigarettes (1R6F). Gas phase radicals differed in polarity with HnB products and conventional cigarettes producing more polar radicals compared to those produced from e-cigs. Free radical production should be considered in evaluating the toxicological profile of nicotine delivery products and identification of the radicals is of paramount importance.



INTRODUCTION

Heated tobacco products, commonly referred to as "heat-not-burn" (HnB) products, are a new category of tobacco products developed by tobacco companies in an effort to produce a less harmful alternative to traditional cigarettes. Specifically, Philip Morris International (PMI) introduced IQOS, British American Tobacco (BAT) introduced Kent Glo, and Japan Tobacco International (JTI) introduced Ploomtech (Figure 1). All of these HnB products began being marketed around the same time in the early 2010s in Japan, and some products are currently sold in over 30 countries.[1] Recently, the IQOS began sales in the U.S.A. in 2019.

HnB products borrow properties of both conventional cigarettes and electronic cigarettes (e-cigs). Conventional cigarettes utilize the burning of tobacco, reaching temperatures of over 900 °C, to volatilize the nicotine and flavors. E-cigs, however, utilize a battery and heating coil to heat a mixture of propylene glycol, glycerin, flavoring, and/or nicotine to deliver the aerosol to the user. The maximum temperature electronic cigarettes can reach depends largely on the device. Newer temperature limited electronic cigarette devices, such as the Juul, limit the maximum temperature to approximately 215 °C.[2] HnB products use electronics to heat tobacco, but at much lower temperatures than with conventional cigarettes, to generate nicotine-containing tobacco vapors. The IQOS consists of a battery-powered heating device with a heating blade that is inserted into a small tobacco stick (Heat Stick) composed of a reconstituted tobacco leaf substrate plug. The heating blade reaches a maximum temperature of 340 °C and for a period of either 6 min or 14 puffs.[3] The Kent Glo consists of a battery-powered heating chamber, where a thin tobacco stick (Neo Stick) composed of reconstituted tobacco leaf substrate is inserted. The heating chamber reaches a temperature of 245 °C for a period of 3.5 min.[4] Both of these devices have been found to produce fewer carbonyls and toxicants, like benzo[a]pyrene, as compared to traditional cigarettes; however, there is limited information on the free radical production of these products.[4−6]

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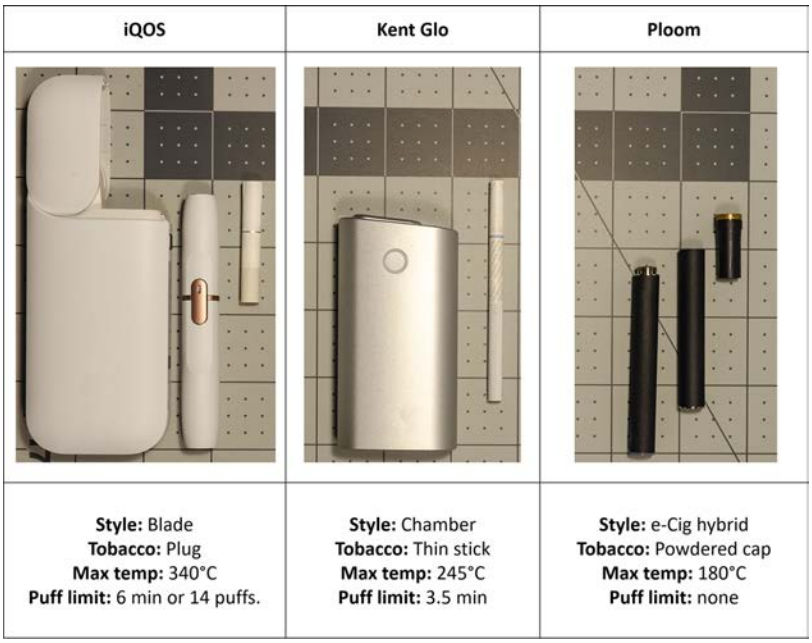

Figure 1. Heat-not-burn products and their associated heatsticks/caps.

As nicotine-containing e-cigs are banned in Japan as a result of the 2010 Pharmaceutical Affairs Act, Japan Tobacco International introduced a hybrid product known as the Ploom.[7] Plooms utilize an e-cig design where propylene glycol and glycerin are heated with a coil, but the resulting vapor is then passed through a chamber containing powdered tobacco allowing tobacco particulates to be picked up by the aerosol and delivered to the user. This product effectively circumvents the ban on nicotine-containing e-cigs in Japan since it is neither an e-cig nor a HnB product. While carbonyl and toxicant have been evaluated in this product, levels of free radical production have not been assessed.[8]

In some parts of the world, HnB products are being marketed by the tobacco industry as a "less harmful and yet "satisfying" alternative to cigarettes to attract smokers. More than 7000 chemicals are generated during the burning of a cigarette, at least 250 of which are considered harmful to humans.[9] Free radicals and oxidants represent a major class of toxicants that are abundant in cigarette smoke. Free radical and oxidant exposure can result in oxidative stress and are thought to play an important role in the development of many tobacco related diseases, such as coronary/vascular disease, chronic obstructive pulmonary disease (COPD), and cancer, as described in the 2010 Surgeon General's Report.[10−12] Cigarette mainstream smoke contains high concentrations of highly reactive free radicals (>10^{16} molecules/puff) in the gas phase as well as longer-lived radicals in the particulate (tar) phase.[13−15] Using electron paramagnetic resonance (EPR) spectroscopy, our laboratory has shown that free radicals are also produced by electronic cigarettes. Further, we found that radical levels are influenced by device characteristics (e.g., voltage, coil resistance), e-liquid composition (e.g., PG, VG, flavor chemicals), and user behaviors (e.g., puff length, puff duration, # of puffs).[16−20] Free radical production by electronic cigarettes were increased in a temperature-dependent manner, increasing nearly 2-fold going from 100 to 300 °C.[18] Juul-like devices have temperature limiting features which prohibit the units from reaching the higher temperatures observed in many mod devices with controllable temperature were found to have low, but significant levels of free radicals.[20] Another key difference between traditional cigarettes and HnB/e-cigarettes is the lack of second-hand (side-stream) smoke. Oxidant damage in the lung because of side-stream smoke is a well-known phenomenon.[21] While particulate phase radicals are thought to play a role, the exact mechanisms of side-stream oxidant damage are not known.

While the exact structures of the e-cig radicals remain unknown, they do appear to be different as compared to conventional cigarettes.[22] As a radical's structure and its electrophilic or nucleophilic nature can dictate the types of molecules it may interact with, assessing basic chemical characteristics may help us identify their potential impacts in the body.[23,24] With the emergence of these new HnB products, we sought to characterize the polarity and determine the levels of free radicals generated from these devices and compare them to other tobacco products.

MATERIALS AND METHODS

Devices and Tobacco. Cigarettes (1R6F), HnB products (IQOS, Kent Glo), the hybrid (Ploom), and e-cigarettes (Juul, SREC, Mod) were tested. Ploom, Kent Glo, and second generation IQOS battery devices were purchased from retailers in Tokyo, Japan in 2018. "Regular" (tan colored pack) flavored Ploom pods, "Regular Bright Tobacco" (blue pack) flavored Neo Sticks, and "Regular" (blue pack) flavored Heat Sticks were also purchased from retailers in Tokyo, Japan in 2018. The Juul and "Classic Tobacco" (brown) pods were purchased from local retailers (Dauphin County, PA, U.S.A.). The U.S. National Institute on Drug Abuse (NIDA) standardized research e-cigarette (SREC) battery and tobacco-flavored e-liquid cartomizer tanks (1.48% w:w nicotine) were purchased from NJOY, LLC. A Wismec Reuleaux RX200S Mod (MyVaporStore.com) mod style e-cigarette was used in consistent temperature mode (240 °C) with a commercially available 0.50Ω stainless steel (SS316) Uwell Crown Coil and stainless steel and glass atomizer tank (MyVaporStore.com). The e-liquid for the mod style device consisted of a standard ratio (60:40) of propylene glycol and glycerine. 1R6F research cigarettes were obtained from the University of Kentucky (Lexington, KY,

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U.S.A.). Cigarettes and the HnB sticks were stored in airtight plastic bags at −20 °C.

Materials. Hexane, glycerol, methanol, phenyl-*N*-*tert*-butylnitrone (PBN), propylene glycol, and *tert*-butylbenzene, 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO), and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) were purchased from Sigma-Aldrich (St. Louis, MO) and used as-received. Cambridge filters pads (CFP) were purchased from Performance Systematix, Inc. (Grand Rapids, MI) and used as-supplied.

Smoke and E-Cigarette Aerosol Generation. Devices were fully charged between each smoking session and tobacco sticks and cigarettes were removed from cold storage and placed in a humidity chamber (60% relative humidity, 22 °C) following ISO 3402:1999, for 48 h before use. Smoke and e-cigarette aerosols were generated using a single-port smoking machine (Human Puff Profile Cigarette Smoking Machine (CSM-HPP), CH Technologies, NJ). As the Juul requires a higher flow rate to activate, all devices were tested with a puff volume of 75.0 mL, a puff duration of 2.5 s, and an interpuff interval of 30 s to effectively compare the devices to each other. This puff profile is based on the CORESTA method with modifications (increased volume and shortened puff duration) made to achieve an adequate flow rate to activate all devices tested.[25] Puffing sessions were determined when the device turned off based on the manufacturer's parameters which resulted in 7 puffs for the Glo and 12 puffs for the IQOS. In the case of the Ploom, Juul, SREC, and the Mod e-cig, 10 puffs were chosen to represent a session. A session for the 1R6F was determined to be 1 cigarette or roughly 11 puffs.

Nicotine Analysis. Total nicotine from smoke/aerosols were trapped onto a CFP located immediately behind the tobacco stick, cigarette, or e-cigarette. Nicotine was extracted from the CFP using 20 mL methanol. Nicotine was then analyzed by gas-chromatography with flame-ionization detection (GC/FID) using an HP 5890 gas chromatograph with separation on an Agilent CP Wax 52 CB column (30 m × 0.25 mm × 0.25 μm) with helium as the carrier gas at a flow rate of 1.2 mL/min. Injector and detector temperatures were held at 240 and 280 °C, respectively. The initial column temperature was 100 °C, held for 1 min, then heated to 240 °C at 10 °C/min, and held for 10 min before returning to initial conditions.

Analysis of Particulate-Phase Radicals. Particulate radicals were trapped on a CFP located immediately behind the tobacco stick, cigarette, or e-cigarette. These CFPs were then analyzed directly using EPR spectroscopy with a Bruker eScan R spectrometer (Bruker-Biospin, Billerica, MA) operating in X-band. EPR parameters were as follows: microwave frequency, 9.7 GHz; modulation frequency, 86.0 kHz; microwave power, 6.00 mW; scan range, 50 G; modulation amplitude, 1.10 G; sweep time, 5.243 s; time constant, 10.240 ms; and conversion time, 10.240 ms. Spin concentrations were determined from the integration of the area under the curve of the EPR signal using WinEPR software (version 0.98, National Institute of Environmental Health Sciences, National Institutes of Health, U.S.A.). Concentrations were determined using TEMPOL standards in methanol pipetted onto CFPs as described previously.[19]

Analysis of Gas-Phase Radicals. The gas-phase of the smoke/aerosols that passed through the CFP was captured in an impinger containing 0.05 M nitrone spin trap, PBN, in 6 mL hexane as previously reported.[18] After each session was completed, the hexane was evaporated under vacuum and the residue was reconstituted in *tert*-butylbenzene, placed into high purity quartz EPR tubes, and subjected to freeze−pump−thaw technique with argon via a Schlenk line.[26] PBN radical adduct derived spectra were measured via EPR with the following parameters: microwave frequency, 9.7 GHz; modulation frequency, 86.0 kHz; microwave power, 2.89 mW; scan range, 60G; modulation amplitude, 1.15 G; sweep time, 10.49 s; time constant, 20.48 ms; and conversion time, 20.48 ms. Quantification of the gas-phase free radicals was done using the second integral and the values were compared against known concentrations of a stable radical standard, TEMPO, as done previously.[17]

Characterization of Gas-Phase Radical. To classify the polarity of the radicals produced by devices, smoke/aerosol was passed through an impinger containing either 20 mL of nonpolar solvent (hexane) or 20 mL of polar solvent (water) before passing to a second impinger containing the PBN solution. The PBN solution was analyzed for radicals as done above.

Statistics. All measurements were done in triplicate. Significant differences ($p < 0.05$) between devices were determined using Brown-Forsythe and Welch ANOVA tests and multiple comparisons done with a two-stage step-up method of Benjamini, Krieger, and Yekutieli via GraphPad Prism (San Diego, CA). Significant differences between unfiltered radicals and the polar and nonpolar radicals within a given device were determined using a one-way ANOVA and a Dunnett's multiple comparisons test.

RESULTS

Gas-Phase and Particulate-Phase Radicals. The research cigarette (1R6F) produced significantly more gas-phase radicals per session with an average of 568 ± 78.3 pmol radicals per puff, nearly 12-fold more than any of the other products tested (Figure 1). Among the other products, the mod e-cigarette device (48 ± 1.8 pmol/puff) and the SREC (40 ± 0.8 pmol/puff) produced significantly more radicals than the HnB products. No differences between the HnB products were seen, however, they produced more radicals than the Juul (5.3 ± 0.5 pmol/puff). Particulate phase radicals were only detected in the 1R6F (744 ± 7.5 pmol/puff) (Figure 2). The variations in radical production were similar to what

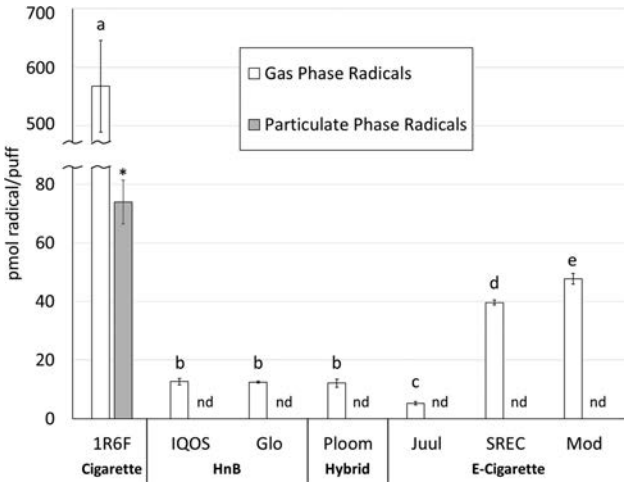

Figure 2. Gas-phase and particulate-phase radicals per puff. Different letters represent statistical differences ($p < 0.05$) between gas-phase radicals. Asterisk (*) represent statistically significant difference ($p < 0.05$) between particulate-phase radicals.

we have found in the past.[17,19,20] Background levels of radicals present in the lab air were also performed but were negligible (0.07−7.75% of measured radical signals) suggesting that radical levels attributable to background/auto-oxidation of PBN were minimal (Supporting Information, SI, Figure 1).

Nicotine. The research cigarette (1R6F) produced significantly more nicotine per puff (190 ± 7.9 μg/puff) than the other products with the exception of the Juul (156 ± 44.6 μg/puff) (Table 1). Of the HnB devices, the IQOS (122 ± 9.6 μg/puff) produced the most nicotine, followed by the Glo (72 ± 10.6 μg/puff) and then the Ploom (18 ± 0 μg/puff). The IQOS, Glo, and SREC (71 ± 8.2 μg/puff) did not differ significantly in their production of nicotine between each other. The nicotine production from the Juul and the IQOS

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Table 1. Gas-Phase Radicals Per Puff[a]

		per puff				
device	puffs	nicotine (μg)	particulate-phase radicals (pmol)	total gas-phase radicals (pmol)[b]	non-polar characteristic gas-phase radicals (pmol)	polar characteristic gas-phase radicals (pmol)
1R6F	11	189.5 ± 7.9[a]	73.9 ± 7.5	567.6 ± 78.3[a]	444.9 ± 86.9*[b] (78%)	9.6 ± 2.8 (2%)
IQOS	12	122.2 ± 9.6[b,c]	nd	12.6 ± 1.1[b]	13.9 ± 0.9*[b] (110%)	6.8 ± 1.6 (54%)
Glo	7	72.1 ± 10.6[b]	nd	12.5 ± 0.3[b]	14.3 ± 2.8*[b] (115%)	8.2 ± 1.9 (66%)
Ploom	10	18.0 ± 0.0[d]	nd	12.1 ± 1.4[b]	11.3 ± 2.2*[b] (93%)	7.0 ± 1.0 (58%)
Juul	10	155.7 ± 44.6[a,b]	nd	5.3 ± 0.5[c]	2.4 ± 1.4 (46%)	5.9 ± 1.1*[b] (113%)
SREC	10	71.0 ± 8.2[b]	nd	39.6 ± 0.8[d]	14.4 ± 0.7 (36%)	39.2 ± 0.9*[b] (99%)
Mod	10	nd	nd	47.8 ± 1.8[e]	19.2 ± 0.3 (40%)	43.3 ± 2.9*[b] (91%)

[a]Different letters represent statistical differences ($p < 0.05$) between products. Asterisks (*) represent statistically significant differences ($p < 0.05$) from the total gas-phase radicals within a product. Non-polar characteristic radicals and polar characteristic radicals are presented in percentages (%) of the total gas-phase radicals below each value. [b]Determined without the use of an impinger upstream of the PBN impinger.

did not differ significantly. The mod e-cig did not produce any nicotine as the e-liquid used was nicotine free.

When radicals were expressed on a per nicotine basis, the research cigarette (1R6F) produced significantly more gas-phase radicals/mg nicotine (2982 ± 251 pmol/mg) and more particulate-phase radicals/mg nicotine (392 ± 61.1 pmol/mg) than any of the other devices (Figure 3). Gas-phase radicals/

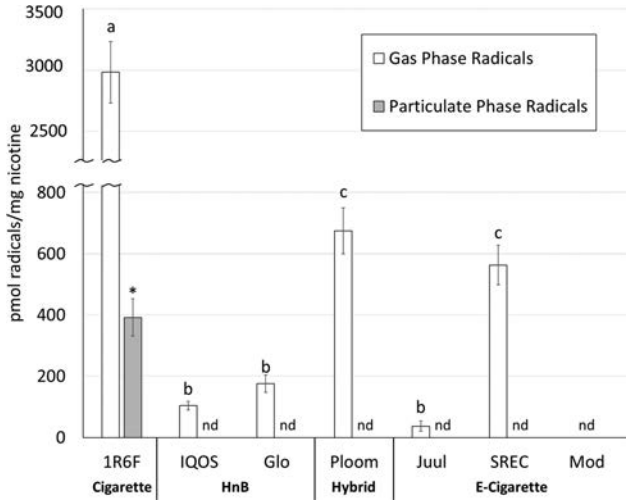

Figure 3. Gas-phase and particulate-phase radicals on a per mg nicotine basis. Different letters represent statistical differences ($p < 0.05$) between gas-phase radicals. Asterisk (*) represent statistically significant difference ($p < 0.05$) between particulate-phase radicals.

mg nicotine from Ploom (675 ± 75.4 pmol/mg) and SREC (563 ± 65.1 pmol/mg) were significantly less than the 1R6F but significantly higher than the other devices. IQOS (104 ± 14 pmol/mg), Glo (176 ± 28 pmol/mg), and Juul (37 ± 16.5 pmol/mg) gas-phase radicals/mg nicotine did not differ statistically from each other. As the mod e-cig did not contain nicotine, it was excluded from this comparison.

Gas-Phase Radical Characterization. After passing the 1R6F cigarette's gas phase through the hexane trap, only 9.6 ± 2.8 pmol radicals per puff were detected, representing a significant loss of over 98% of the radicals as compared to the control without a trap. While a small decrease was seen with the water trap, it was not statistically significant (Table 1). All the HnB products also showed significant decreases (IQOS 6.8 ± 1.6, Glo 8.2 ± 1.9, Ploom 7.0 ± 1.0 pmol/puff) in radicals when vapor was passed through the hexane trap. The HnB radicals were unaffected by the water trap. The e-cigarette devices showed the opposite trend (Juul 2.4 ± 1.4, SREC 14.4 ± 0.7, mod e-cig 19.2 ± 0.3 pmol/puff) with significant decreases in radical detection only after passing through the water traps. The e-cigarette device radicals were unaffected by the hexane trap.

DISCUSSION

Conventional cigarettes have been known to produce reactive free radicals as well as numerous other toxicants since the 1980s; however, effective strategies to reduce toxicant exposure and related harm have progressed slowly.[27] Over 30 years later, new nicotine delivery devices, including e-cigs and HnB, have appeared on the market with the hope of reducing harm by decreasing the production of toxic combustion related byproducts. Here, we show that these devices greatly reduce a user's exposure to highly reactive free radicals, though exposure levels are still much higher than those obtained from other environmental sources. Similarly, these devices also appear to produce fewer carbonyls and tobacco specific nitrosamines (TSNAs) such as NNN, NAT, NAB, and NNK.[5,28]

The levels of gas phase free radicals detected in HnB products in our study are similar to radical levels produced by e-cigs and significantly less than conventional cigarettes. We also found that both e-cigs and HnB products produce levels of particulate phase radicals well below the limit of detection suggesting that harm associated with exposure to these more stable radicals found in the particulate phase of conventional cigarette smoke may be greatly reduced as has been found by others.[6] Larger particles (>3 μM) have been found to deposit largely in the nasal, pharyngeal, and laryngeal tracts while smaller particles (<1 μM) often reach and deposit along the tracheobronchial and alveolar tracts.[29] The presence of particulate phase radicals in conventional cigarettes and absence in the other products suggests that radical deposition in the lung may be different and potentially influence smoking-related disease outcomes. Previously, we found that puff volume was the only factor that significantly influenced radical production in conventional cigarettes.[30] As the puffing topography of how these new products are used remains unknown and we only tested a singly puffing profile, additional puffing parameters may indeed influence radical production from these new products. As highlighted previously, the use of EPR remains as the only method to accurately quantify radicals as other methods that use fluorescent dyes, such as 2′,7′-dichlorofluorescein diacetate, to measure "reactive oxygen

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species" can provide inaccurate and potentially misleading results.[31] Thus, we stress that only EPR generated data should be considered when assessing potential harm resulting from free radical producing devices, particularly regarding tobacco regulation considerations.

Free radical polarity or the ability of radicals to react with polar or nonpolar agents are important factors affecting the potential biological targets of free radical attack. For example, acetonyl radicals are electrophilic in nature while benzoyl radicals are largely nucleophilic suggesting that they may have different targets within the body.[23,24] However, there is a paucity of data regarding the specific biological targets of free radicals derived from cigarette smoke or e-cigarette/HnB aerosols, in part due to the lack of information regarding the chemical nature of the radicals produced. To begin to understand the nature of radical species produced by different tobacco products, we conducted exploratory experiments to determine the interaction of these radicals with solvents of differing polarity. On the basis of our findings, it appears that free radicals produced from both conventional cigarettes and HnB devices are more hydrophobic in nature, as they are effectively removed by passing through a nonpolar (hexane) trap, while e-cigarette radicals are more polar in nature, as they were effectively removed by passing through a polar (water) trap. The loss of radicals by either a hexane or water trap is likely a result of interactions with the polar/nonpolar solvents as observed previously for cigarette smoke or simply a result of the polarity/solubility of the radicals themselves.[32] While it is possible that different components of cigarette, HnB, and e-cigarette smoke/vapor modulate the radical trapping efficiency, this seems unlikely due to the differences seen between the different polar/nonpolar traps. The differing nature of the e-cigarette radicals compared to the HnB and conventional cigarettes could result in different toxic effects and targets of attack within the upper aerodigestive tract and lungs of the user. While a recent study found that HnB products and e-cigarettes also produce a carbon-centered radical not found in conventional cigarettes, future studies are required to provide data on the chemical identity of these radicals as well as the types of free radical damage observed in exposure models with the different products.[6]

In some parts of the world, HnB products are promoted by tobacco companies as a less harmful alternative to traditional cigarettes. However, none of the tobacco companies claim reduced morbidity and mortality by the use of these lower toxicant products. In the U.S., modified risk claims cannot be part of advertising and/or marketing unless expressly authorized by the Food and Drug Administration (FDA). None of the products tested, except for the Juul, produced comparable amounts of nicotine to the 1R6F research cigarette on a per puff basis. This suggests that while they may be producing fewer radicals, they may not be an adequate nicotine replacement device for a smoker. As a result, a user of a lower nicotine producing device, such as the Ploom or the SREC, would need to take more puffs to satisfy their craving, exposing them to higher levels of free radicals. Our present results where free radical delivery is expressed on a nicotine basis support this notion as the relative radical delivery per nicotine is greater for these products and only 4.5- to 5.3-fold lower than levels found in combustible cigarettes.

Besides the nicotine, which is addictive, HnB products appear to still produce some of the same types of harmful toxicants found in cigarettes (e.g., reactive free radicals), albeit at lower levels. The toxicological harm associated with long-term exposure to the type and levels of free radicals in HnB remains to be investigated. Free radical exposure, regardless of the level, poses a potentially significant health risk, as these highly reactive species can damage critical cellular pathways which can lead to carcinogenesis and other disorders.[10−12]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.0c00088.

Background radical measurements (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

BAT, British American Tobacco; CFP, Cambridge filters pads; COPD, chronic obstructive pulmonary disease; CSM-HPP,

Human Puff Profile Cigarette Smoking Machine; EPR, electron paramagnetic resonance; FDA, Food and Drug Administration; GC/FID, gas-chromatography with flame-ionization detection; HnB, heat-not-burn; JTI, Japan Tobacco International; NAB, *N*-nitrosoanabasine; NAT, *N*′-nitrosoanatabine; NIDA, National Institute on Drug Abuse; NNK, nicotine-derived nitrosamine ketone; NNN, *N*-nitrosonornicotine; PBN, phenyl-*N*-*tert*-butylnitrone; PG, propylene glycol; PMI, Philip Morris International; SREC, standardized research e-cigarette; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxyl; TEMPOL, 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl; TSNAs, tobacco specific nitrosamines; VG, vegetable glycerine

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